



Supplementary Figure 4. Concentration independence of FRET from purified sstFRET protein and 1:1 Venus+Cerulean. (A) Plot of the FRET ratio of the Venus+Cerulean mixture versus acceptor channel intensity. The FRET ratio was calculated as described in methods. As acceptor channel intensity gradually decreased, the FRET ratio remained constant until the acceptor channel intensity approached zero. The green line covers the intensity range where the means of FRET ratio is 1.01. (B) Plotting the FRET ratio of sstFRET protein solution versus acceptor channel intensity. Only when the acceptor channel intensity reached 500 did the FRET ratio fall to a lower value. The green line covers the intensity range where the FRET ratio was constant. The mean of the sstFRET FRET ratio in solution was 1.38, which is in line with the value obtained from living cells (N>3 for both preparations).